


Rhodamine 6G efflux assay

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 An abbreviated version of this protocol was published in Scientific Reports in Mar 2019

Gene Duplication Associated with Increased Fluconazole Tolerance in *Candida auris* cells of Advanced Generational Age

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Detailed protocol

Rhodamine 6G efflux Assay:

1. In preparation for the assay, a single colony of each isolate was suspended in 5 ml Synthetic Dextrose (SD), (6.7 g Bacto Yeast Nitrogen Base without amino acids and 20 g glucose/litre) medium with 2% glucose and incubated at 37°C with shaking at 180 rpm.
2. Next day, 0.1 OD of cells were exponentially grown (6-7 h) in SD medium with 2% glucose at 37°C with shaking at 180 rpm.
3. 10^8 exponentially growing cells from each isolate were then washed three times with 1× phosphate-buffered saline (PBS; pH 7.4), starved for 2 h in 1× PBS containing 5mM deoxy-glucose, and incubated at 37°C.
4. 5 µl of a 10 mM stock of Rhodamine 6G (R6G) was added to 5 ml of a starved cell suspension to a final R6G concentration of 10 µM. The 10mM stock solution of R6G was prepared in 100% ethanol
5. The cells were then incubated at 37°C for 30 mins.
6. The cells were then pelleted and washed once with 1X PBS.
7. The washed cells were resuspended in 1× PBS plus 2% glucose to initiate efflux.
8. 200µl samples were then collected at 0-, 30-, and 60-mins intervals.
9. The collected samples were pelleted, and the supernatants were pipetted into black microtiter 96-well plate.
10. The fluorescence of the supernatants was measured at excitation and emission wavelengths of 525 nm and 555 nm respectively.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fries, B. and Bhattacharya, S. (2021). Rhodamine 6G efflux assay. Bio-protocol Preprint. bio-protocol.org/prep1243.
2. Bhattacharya, S., Holowka, T., Orner, E. P. and Fries, B. C.(2019). Gene Duplication Associated with Increased Fluconazole Tolerance in *Candida auris* cells of Advanced Generational Age. Scientific Reports 9. DOI: [10.1038/s41598-019-41513-6](https://doi.org/10.1038/s41598-019-41513-6)

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